Water Absorption of Proteins. VI. Effect of Guanidino Groups in Casein[1]

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The vapor phase water absorption isotherms[2] of the amino groups in casein and of the peptide groups of some long polyglycine molecules show that these polar groups are specific sorptive sites over the entire range of relative humidities. The basic nature of the guanidino group indicated that it might also be a specific site for water absorption. Therefore, guanidino groups have been introduced into casein by the reaction of S-methylisothiourea with the free amino groups in alkaline solution. Several casein derivatives containing various amounts of substituted guanidino groups have been prepared in this manner.

The vapor phase water absorption isotherms of these samples were obtained as in the previously described studies.[2] The absorptions were calculated for a 1000-g. content of casein to eliminate the dilution effect due to the varying weight of guanidino group in each sample. The absorption of the substituted samples were subtracted from the control sample and the results listed in Table I.

Table I

Decrease in Absorption Due to Substitution

Relative humidity, %	Sorption of control, g./1000 g.	Decreased absorption, g./1000 g. casein, by samples 1	2	2A[a]
11.8	35	0	0	9
31.4	69	1	−1	16
50.9	97	0	0	23
75.1	146	2	1	32
83.6	174	6	5	40

[a] Calculated values for decreased absorptions due to the removal of the same number of amino groups as were substituted in sample 2.

The data listed for sample 2A are the calculated water absorption values for the same number of free amino groups[2] in casein as have been substituted in derivative 2. The absorption of the control sample was equal to the absorption of the same original casein which had not been treated with the alkali. This shows that there was no significant change in the casein due to this treatment. These data show that the vapor phase water absorption of the guanidino groups produced from the amino groups is not significantly different from the absorption of the free amino groups themselves.

These introduced guanidino groups differ from the naturally occurring guanidino groups in proteins only in that they are one methylene group further removed from the peptide chain of which they are side groups. This, however, would not be expected to cause any great difference in the polarity or water absorbing capacity of these groups. The 4.1 g. of arginine naturally present in 100 g. of casein[3] contains 0.0235 mole of guanidino groups. Therefore, the guanidino groups naturally present in casein will account for an average of 8.7% of the total water absorption of casein between 30 and 85% relative humidity and may account for as high as 12% at lower humidities.

The almost constant fractions of the total water absorption at all relative humidities which have been found to be due to the amino, peptide and guanidino groups show that these polar groups which comprise only a few per cent. of the total protein must be considered in any theoretical analysis of the sorption phenomena. It is consistent with the polarization theory of Bradley which we have shown fits the data for the water absorption of proteins and other high polymers.[4]

Experimental

Preparation of Guanidinated Caseins.[5]—Twenty-five grams of high nitrogen casein was swelled for one hour in 100 ml. of water. Then 200 ml. of water and 210 ml. of concd. ammonium hydroxide were added, and the mixture was stirred until the casein dissolved. S-Methylisothiourea sulfate (0.0, 4.0 or 12.0 g.)[6] in a small amount of water was added and the mixture was stored at 5° for three days. The solution was then transferred to a viscose tube and dialyzed against running distilled water until the dialysate was free of ammonia when tested with Nessler reagent. The solution was brought to *p*H 4.5 with hydrochloric acid. The precipitate was centrifuged and washed as in the previously described method. These products were air-dried, and ground to pass a 60-mesh screen.

The products were analyzed for total nitrogen by the Kjeldahl method, and for amino nitrogen by the Doherty and Ogg[7] modification of the Van Slyke method. Arginine was determined by the Sakaguchi method on a total hydrolysate of the protein; the reagents and conditions developed by MacPherson[8] were used. The presence of the other amino acids in the hydrolysate results in high arginine values by this procedure but the values obtained on a more extended series are sufficient to show that we get a proportionate increase in guanidine groups as the free amino groups are substituted. These analyses for the control and samples 1 and 2, respectively, are: total nitrogen, 14.97, 15.66 and 16.38; amino nitrogen, 0.91, 0.37 and 0.06; and apparent arginine, 5.53, —, 18.97.

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